

## MRI Evaluation of Selective Disruption of the Blood-Brain Barrier by Photochemical Internalization

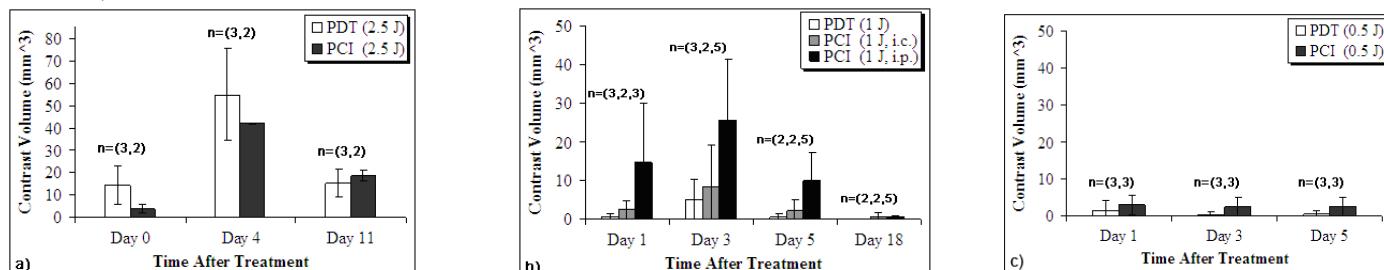
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**Introduction:** Failure to eradicate infiltrating glioma cells using conventional treatment regimens results in tumor recurrence and is responsible for the dismal prognosis of patients with glioblastoma multiforme (GBM). Migrating glioma cells are protected by the blood-brain barrier (BBB) that prevents the delivery of most anti-cancer agents. Photochemical internalization (PCI) is being developed to selectively disrupt the BBB since PCI causes the release of membrane-impermeable molecules into the cytosol of target cells [1]. Disruption of the BBB permits access of anti-cancer drugs to effectively target infiltrating tumor cells, and potentially improves the treatment effectiveness for malignant gliomas. BBB disruption was evaluated using MRI after initiating photodynamic therapy (PDT) alone [2] and in combination with PCI in a rat model.

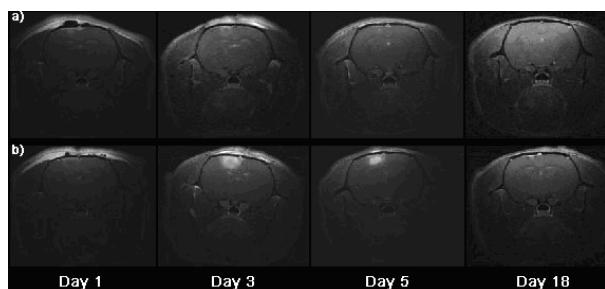
**Materials and Methods:** PCI treatment, coupling the macromolecule *Clostridium perfringens* (*Cl p*) epsilon prototoxin with AlPcS<sub>2a</sub>-PDT, was performed on non-tumor bearing inbred Fischer rats. An AlPcS<sub>2a</sub> concentration of 1 mg/kg combined with light fluences of 2.5, 1 or 0.5 J and two *Cl p* prototoxin delivery methods, intraperitoneal (i.p.) and intracranial (i.c.) were evaluated for the extent of BBB disruption in the group of animals receiving PCI treatment. The control group of animals received an AlPcS<sub>2a</sub>-PDT only treatment using the same combination of AlPcS<sub>2a</sub> concentration and light fluences used in PCI.

Treated animals were anesthetized with isoflurane and imaged in a Bruker 7.0 T/20 MRI. T<sub>2</sub>-weighted MR images (TR: 4200 ms; TE: 36 ms) were acquired to evaluate brain edema, a marker for BBB disruption, induced by PDT-only or PCI treatments. T<sub>1</sub>-weighted (TR: 700 ms; TE: 14 ms) post-contrast MRIs were acquired 15-20 min after injection of MultiHance (0.8 ml i.p.). Contrast enhancement evident on T<sub>1</sub>-weighted images was taken as direct evidence of BBB disruption induced by the corresponding treatment. The qualitative analyses of contrast and edema volumes were performed using MIPAV (Medical Image Processing, Analysis & Visualization) software.



**Figure 1.** Contrast volumes induced by PDT and PCI at a light fluence of a) 2.5 J; b) 1 J and c) 0.5 J. The animals received 1 mg/kg AlPcS<sub>2a</sub>. The PCI group of animals also received an i.p. or i.c. injection of *Cl p* prototoxin. The n values listed above each data set indicate the number of animals used to generate the corresponding data bars.

**Results and Discussion:** The PCI effect in rat brain was found to be dependent on light fluence, photosensitizer concentration, *Cl p* prototoxin concentration and administration route (Fig. 1). Selective disruption of the BBB by PCI was observed for intraperitoneal administration of 1:100 stock dilutions of *Cl p* prototoxin and photosensitizer concentrations and light fluences of 1 mg/kg and 1 J, respectively (Figs. 1 and 2). Single modality treatments consisting of PDT or *Cl p* resulted in only minimal damage to the BBB.



**Figure 2.** Longitudinal comparison of T<sub>1</sub>-weighted post-contrast MRI scans for a PDT treated animal (top row) and a PCI treated animal (bottom row) receiving an i.p. injection of *Cl p* prototoxin. Both received a light fluence of 1 J.

Both PDT and PCI treatments induced timely, localized BBB disruptions. However, the PCI effect induced a much greater extent of BBB disruption than PDT for a light fluence of 1 J (Figs. 1b and 2). The extent of BBB opening peaked on day 3 and was completely restored by day 18 after PCI. Higher light fluences are required for PDT compared to PCI to achieve similar effects on the BBB.

Unfortunately, these higher light fluences increase the potential for edema formation and hence, morbidity and mortality. Therefore, under the appropriate conditions, PCI would appear to be a safe alternative to PDT for selective disruption of the BBB.

**References:** 1. K. Berg et al., Curr Pharm Biotechnol 2007; 8(6):362-72. 2. H. Hirschberg et al., Lasers Surg Med. 2008;40(8):535-42.